CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
ACEQUINOCYL TECHNICAL (formerly AKD-2023)

Chemical Code # 5801, Tolerance # 52893
SB 950 # N/A
Original date:8/30/02
Revised date: 3/6/03

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, possible adverse effect
Chronic toxicity, dog:	No data gap, no adverse effect
Oncogenicity, rat:	No data gap, no oncogenicity effect
Oncogenicity, mouse:	No data gap, no adverse effect
Reproduction, rat:	No data gap, possible adverse effect
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.

All record numbers for the above study types through Document No. 52893-0054 (Record No. 202611) were examined.

In the 1-liners below:
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
indicates a study on file but not yet reviewed.

File name: T191890A
Reviews by Aldous, Corlett, Gee, Leung and Moore as of 3/6/03

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may identify additional effects.

NOTE: A metabolism study report (DPR Document No. 52893-032, Record No.184426, page 38) noted that the principal identified urinary metabolites (AKM 14 and AKM 15) have a red color, which may explain red discoloration of excreta in various animal experiments. Both metabolites are common components of bile, and AKM 15 persists in small amounts in feces. Thus, in the absence of information to the contrary, staining of excreta when noted below should be considered as an indication of exposure, but not as a toxic effect. Aldous, 8/19/02.

COMBINED, RAT

****52893-030 184421** Inoue, H., "Chronic feeding and oncogenicity study with AKD-2023 Technical in rats," Biosafety Research Center, Shizuoka, Japan, 3/5/98 (translated). Laboratory Report # 3953. Diets contained 0, 50, 200, 800, and 1600 ppm acequinocyl (97.1%). Reported mean test substance intakes were 2.25, 9.02, 36.4, and 74.0 mg/kg/day in males and 2.92, 11.6, 46.3, and 93.6 mg/kg/day in females. Fifty F344 rats/sex/group were assigned to the lifetime (2-yr) study. An additional 10/sex/group constituted interim sacrifice groups, terminated at weeks 26, 52, and 78. The latter rats were used for clinical chemistry, hematology, and urinalysis. Interim rats were all examined grossly, however histopathology of interim groups was limited to the 1-yr sacrifice rats. NOEL = 50 ppm (dose-related increases in "hypertrophy of the eyeball" in 200 ppm males). "Hypertrophy of the eyeball" and "corneal abnormality" were common findings in 800 and 1600 ppm males. "Hypertrophy of the eyeball" was a treatment response in 1600 ppm females. Findings of red-brown to dark-red urine were noted in both sexes at 800 to 1600 ppm. This may represent a metabolite of acequinocyl, which is a colored naphthoquinone similar to Vitamin K. Other definitive treatment effects were limited to 1600 ppm males, such as body weight decrements and hematology changes, which were generally only remarkable during the first year [increased platelet counts, increased prothrombin time, and increased activated partial thromboplastin time (APTT)]. Ophthalmology was only performed at 2-yr termination, which was beyond the optimal time to distinguish ocular treatment effects. Nevertheless, high dose rats had small increases of cases in which the ocular fundus could not be visualized. **Acceptable**, with responses in the eyes as "**possible adverse effects**." Aldous, 7/29/02.

CHRONIC TOXICITY, RAT

See "COMBINED, RAT" section, above.

CHRONIC TOXICITY, DOG

52893-028 184419 Waterson, L. A., "AKD-2023 Technical: Toxicity to dogs by repeated oral administration for 52 weeks," Huntingdon Life Sciences Ltd., Cambridgeshire, 7/23/96. Lab Project ID AGK 30/952818. Four beagles/sex/group were dosed by capsule at 0, 5, 20, 80, or 320 mg/kg/day for 1 year with Acequinocyl Technical (formerly AKD-2023), purity 97.1%, in a chronic study. NOEL = 20 mg/kg/day [slightly (not significantly) elevated platelet counts at most time periods in both sexes at 80-320 mg/kg/day]. Common findings at 320 mg/kg/day included deaths of one/sex, associated with "poor general condition;" elevated reticulocyte counts in females; reduced serum protein levels (reduction of globulin levels in both sexes, and of albumin levels in males); and general reduction in urinary volume (M & F) with associated elevations in urinary specific gravity and protein concentration. Study is **acceptable, with **no adverse effects** (mortalities were limited to a comparatively high dose level, without evidence of sensitive endpoints for toxicity). Stained feces were evident at 80 to 320 mg/kg/day, and colored sawdust (red, orange, or brown) was seen at 20 to 320 mg/kg/day. These observations

were not associated with apparent toxicity, and appear to reflect a metabolite of the yellow-colored test article. Aldous, 7/22/02.

ONCOGENICITY, RAT

See "COMBINED, RAT" section, above.

ONCOGENICITY, MOUSE

52893-029 184420 Waterson, L. A., "AKD-2023 Technical: potential tumorigenic and toxic effects in prolonged dietary administration to mice," Huntingdon Life Sciences Ltd., Cambridgeshire, 2/27/94. Laboratory Project ID: AGK 29/961180. Acequinocyl Technical (formerly AKD-2023), purity 97.1%, was administered in diets of fifty Crl: CD-1(ICR)BR mice/sex/group at 0, 20, 50, 150, or 500 ppm in a lifetime (80 wk) study. An additional 20/sex/group were assigned to a satellite study (54 wk duration). Essential features of an oncogenicity study were included in design, plus some features of a combined study (excluding ophthalmology). NOEL < 20 ppm (lowest dose tested: equal to 2.7 and 3.5 mg/kg/day in M and F, respectively), based on "brown pigmented cells, perivascular or sinusoidal." Also, elevated incidence of fatty hepatocytes, periportal or generalized, was found in both sexes at 50 to 500 ppm. Associated findings were increased liver weights (typically statistically significant at 150 and 500 ppm in both sexes), and slight increases in "pale" livers or "pale areas" within livers at these dose levels. Perivascular inflammatory cell aggregation was substantially elevated in livers of 150 and 500 ppm females and in 500 ppm males, and appears to be a response to hepatocellular toxicity. Glomerular amyloidosis was statistically elevated in 150 and 500 ppm males of the lifetime study, and may represent a treatment effect. Darkened urine (yellow to orange-brown) was a consistent high dose finding in both sexes. Study is **acceptable, with **no adverse effects**. Aldous, 7/29/02.

REPRODUCTION, RAT

****52893-027 184418** Osterburg, I., "AKD-2023 Technical: two generation oral (dietary administration) reproduction toxicity study in the rat (one litter per generation)," Corning Hazleton GmbH, Münster, Germany, 1/21/97. Laboratory Study # 659-004. Two generations of Crl: CD (SD)BR rats (25/sex/group, 1 mating/generation) were dosed in diet with Acequinocyl Technical, purity 97.1%, at 0, 100, 800, or 1500 ppm in a reproduction study. Investigators assessed delivery and litter data, estrous cycle patterns, sperm motility and morphology, developmental stage attainment, pupillary reflex, and auditory startle response. Weaned pups selected to become F1 parents were additionally evaluated in open field tests and a water maze test. Achieved dosages were 7.3, 59, and 111 mg/kg/day in F0 males and 8.7, 69, and 134 mg/kg/day in F0 (pre-mating period) females [doses marginally higher in F1 rats]. Reproduction and neonatal viability NOEL = 1500 ppm (HDT). "Parental" NOEL = 100 ppm (hemorrhages, particularly in head and extremities, with some swelling of these tissues). All signs were most prominent during the first several days after weaning, when pups began to consume exclusively solid food. Many 1500 ppm weanlings and a few at 800 ppm (F1 generation only) died between post-natal days 22 and 35. Clinical signs and necropsies confirmed frequent instances of subcutaneous bleeding of extremities and head , blood-filled cranial or abdominal cavities, cerebral hemorrhage, eyes swollen or hemorrhaged, and some other soft tissue changes at 1500 ppm. Study is **acceptable** with some deficiencies. Some developmental physical and functional delays were noted in 1500 ppm juvenile rats. These were likely to be attributable to hemorrhage-related stress, and are certainly secondary in importance to the same. The hemorrhage/bleeding and associated mortalities are "**possible adverse effects**", considering the prevalence in juvenile rats. Aldous, 7/31/02.

52893-037 187917 Irvine, L. F. H., "AKD-2023 Technical: Dietary rat reproductive dose ranging study," Toxicol Laboratories Ltd., Ledbury, Herefordshire, England, 1/19/95. Report No. AKJ/62/R. Key elements of the investigator's summary follow. Groups of 8 pairs of CD rats/group were dosed in diet with 0, 250, 1000, 2000, or 2500 ppm acequinocyl technical for 4 weeks prior to mating until 2 weeks after mating (M) or until weaning at day 21 of lactation (F). Offspring were maintained on treatment until 7 days after weaning (i.e. day 28 post-partum). A dose-related orange staining of the fur was observed at 2000 to 2500 ppm. One 2500 ppm dam had a unilateral uterine hemorrhage with associated adhesions (possibly treatment-related). Reproductive parameters appeared normal in all groups. Pups in these same groups suffered high incidences of lethargy and hemorrhage beginning immediately after weaning, and until premature sacrifice at postpartum days 22 to 24. Very limited possible hemorrhaging (purple discoloration of forelimbs or forepaws) in the 1000 ppm pups was noted after weaning, but was not confirmed at day 28 sacrifice. This study justifies the dose levels used in the primary study, above. Aldous, 8/19/02 (no DPR worksheet).

TERATOLOGY, RAT

52893-026, -0054; 184417, 202611; Irvine, L. F. H., "AKD-2023 Technical: Oral (gavage) rat developmental toxicity study," Toxicol Laboratories Ltd., Ledbury, Herefordshire, England, 2/6/95. Report No. AKJ/63/94. Twenty-five mated Crl:CD®(SD) BR dams/group were dosed by gavage in 10 ml/kg 1% aq. methylcellulose vehicle with 0, 50, 150, 500, or 750 mg/kg/day Acequinocyl Technical (formerly AKD-2023) during gestation days 7-17 in a developmental toxicity study. The highest dose proved excessive. Although all 750 mg/kg/day dams were taken off treatment during days 10 to 13, four of this group were killed *in extremis* (as also was one dam at 500 mg/kg/day on day 17). Common signs in decedents included blood-staining of vaginal opening, pale body, pale eyes, hypoactivity, piloerection, slow or irregular breathing, intra-uterine hemorrhage, and blood-stained stomach and/or intestinal contents. Of the remaining 20 pregnant 750 mg/kg/day dams, one suffered 100% early resorptions, two had all but one fetus resorbed, and four surviving dams had red vaginal discharge between days 13 to 17. Maternal NOEL = 150 mg/kg/day, based on these signs. Developmental toxicity NOEL = 150 mg/kg/day, based in part on incomplete ossification of parietals, reduced numbers of caudal centra, and increased incidence of thoracic centra bilobed or bipartite. Two high dose fetuses had malformations of the tail ("short" tail in one fetus and "filamentous" tail in another of a separate litter). Also, "filamentous" tail was found once in the 500 mg/kg/day group. Background incidences of tail malformations are low, and a treatment effect is plausible. Incidences of the latter were sufficiently marginal and evidences of maternal toxicity at those dose levels were sufficiently well-defined that the data do not warrant designation of a "possible adverse effect." **No adverse effect indicated**. Study previously unacceptable (no stability data for suspensions under room temperature storage conditions). (Aldous, 8/29/02); Stability data submitted in vol. 52893-0054, rec. no. 202611 indicate that the dosing preparations are stable up to 8 days at room temperature; **Study acceptable.** (Moore, 3/6/03)

52893-037 187916 Irvine, L. F. H., "AKD-2023 Technical: Oral (gavage) rat developmental toxicity dose ranging study," Toxicol Laboratories Ltd., Ledbury, Herefordshire, England, 1/19/95. Report No. AKJ/60/R. Key elements of the investigator's summary follow. Eight SD dams/group were dosed by gavage with 30, 150, 250, or 300 mg/kg/day acequinocyl in 1% aq. methylcellulose vehicle during gestation days 7-17. Dams were observed during gestation, then sacrificed at day 20, when dams and fetuses were examined. No effects were observed in this range. A group of dams was similarly dosed at 500 mg/kg/day. These dams had modest (not statistically significant) body weight gain decrements. Based on these results, a range up to 750 mg/kg/day was selected for the primary study. Aldous 8/19/02. (No DPR worksheet).

TERATOLOGY, RABBIT

52893-026,-0054; 184416, 202611; Irvine, L. F. H., "AKD-2023 Technical: Oral (gavage) rabbit developmental toxicity study," Toxicol Laboratories Ltd., Ledbury, Herefordshire, England, 2/17/95. Report No. AKJ/64/94. Eighteen timed-pregnant NZW does/group were dosed by gavage with 0, 30, 60, or 120 mg/kg/day Acequinocyl Technical (formerly AKD-2023) in 1% aq. methylcellulose vehicle (4 ml/kg) during gestation days 6-18 in a developmental toxicity study. Maternal NOEL = 60 mg/kg/day (moribund sacrifices of 5/17 pregnant 120 mg/kg/day does following signs of "reduced quantity of feces" or "absent feces" and/or moderate amount of red liquid on tray liner. Fetal NOEL = 60 mg/kg/day (increased incidence of extra 13th rib). **No adverse effects indicated** (minor increase in a common variation at a maternally toxic dose); Study previously unacceptable (no stability data for suspensions under room temperature storage conditions). (Aldous, 8/01/02); Stability data submitted in vol. 52893-0054, rec. no. 202611 indicate that the dosing preparations are stable up to 8 days at room temperature; **Study acceptable.** (Moore, 3/6/03)

52893-037 187915 Irvine, L. F. H., "AKD-2023 Technical: Oral (gavage) rabbit developmental toxicity dose ranging study," Toxicol Laboratories Ltd., Ledbury, Herefordshire, England, 1/19/95. Report No. AKJ/61/R. Key elements of the investigator's summary follow. In the first stage, 5 non-pregnant NZW rabbits were dosed with progressively higher acequinocyl technical doses (gavage, 1% aq. methylcellulose vehicle) in steps lasting 5 days each, beginning at 50 mg/kg/day, and progressing through 100, 150, 250, and finally 350 mg/kg/day. Red-stained urine was observed at 150 mg/kg/day and above. One rabbit died at 250 mg/kg/day, and all four rabbits surviving to the 350 mg/kg/day dosing period showed reduced food consumption, reduced body weights, and reduced fecal output. Based on these results, a group of 5 non-pregnant females was dosed for 14 days at 300 mg/kg/day. Four of these had reduced fecal output, 3 had consistently reduced food consumption, 3 had body weight decrements, and 2 were killed *in extremis* by day 10. Some treated females had reduced RBC parameters, increased reticulocyte counts, and various clinical chemistry changes. One of these appeared to have hemorrhages in eyes and thymus, plus other gross abnormalities. These tests determined the range for a pilot developmental toxicity study. Groups of 8 pregnant does were dosed at 0, 30, 60, 120, or 240 mg/kg/day during gestation days 6-18. These were necropsied at day 28, and fetuses were examined externally. All 240 mg/kg/day does were sacrificed prematurely following body weight losses, reduced food consumption, reduced or loose or liquid feces, and/or evidence of vaginal hemorrhage. There were high numbers of resorptions in these does. At 120 mg/kg/day there was one doe with red liquid in the cage tray (presumed not merely metabolite-stained urine), but there were no statistically significant changes indicative of a treatment effect. Based on these data, the dose range used in the primary study (above) was well-selected. Aldous, 8/22/02. (No DPR worksheet).

GENE MUTATION

** 031 184422 Nakajima, N. "Reverse Mutation Assay of AKD-2023 Technical." (Biosafety Research Center, Shizuoka, Japan, Experiment No. 2004 (154-002), Report No. 2562, February 9, 1998.) AKD-2023 Technical (96.5%) was assayed at 9.77 to 2,500 :g/plate in the presence and absence of metabolic activation (rat liver microsomes) for the potential to induce reverse mutations in *Salmonella typhimurium* strains TA 100, TA 98, TA 1535 and TA 1537 and at 5000 :g/plate with *Escherichia coli* WP2 *uvrA*. A 20 minute pre-incubation step was used before plating. After plating, exposure was at 37/ for 48 hours. There were triplicate plates per concentration with two independent trials. A precipitate was noted at higher concentrations. AKD-2023 Technical, under study conditions, did not increase the frequency of revertants. Positive controls were functional. **ACCEPTABLE**. (Kishiyama and Gee, 7/30/02).

** 031 184423 Reeve, L. "AKD-2023: Mutation at The Thymidine Kinase (*tk*) Locus of

Mouse Lymphoma L5178Y Cells (MLA) Using the Microtitre® Fluctuation Technique". (Covance Laboratories Limited, North Yorkshire, England, CLE Study Number 619/47, Report No. 619/47-D5140, December 17, 1998.) AKD-2023 (97.1%) was tested at concentrations of 0, and 10 to 320 :g/ml in the absence and 10 to 280 :g/ml in the presence of S-9 mix for the potential to induce mutation at the *tk* locus in mouse lymphoma L5178Y cells. There were duplicate cultures per concentration in each trial, three trials without activation and two trials with activation. Exposure time was for 3 hours followed by a 2-day expression period. Following expression, cell dilutions were plated in 96-well microtitre plates, 4 plates per original culture. Viability was determined after treatment and after expression. In trial 2 in the absence of activation, there were statistically significant increases in the mutant frequency at several concentrations. This result, however, was not reproducible and the MF values were close to the historical control values. Also, the control frequency in that trial was lower than in any other trial, with or without activation. There were no indications of a positive result with activation. In those cultures with significant results, the proportion of large and small colonies was similar to solvent controls. AKD-2023 was considered overall as not mutagenic. **ACCEPTABLE**. (Kishiyama and Gee, 8/1/02)

CHROMOSOME EFFECTS

** 031 184424 Nakajima, N. "Chromosomal Aberration Test on CHL Cells Treated with AKD-Technical ." (Biosafety Research Center, Shizuoka, Japan, Experiment No. 2005 (154-003), Report No. 2621, February 9, 1998.) AKD-2023 Technical (96.5%) was assayed at concentrations of 150 to 1,200 :g/ml in the absence of S-9 Mix for 24 and 48 hours exposure and at 481 to 3850 :g/ml in the presence of S-9 Mix (rat liver) with a 6- hour exposure (plus 18 hours) for the potential to induce chromosomal aberrations using Chinese hamster lung fibroblasts. There were duplicate cultures per concentration with 100 cells scored per culture. Mitotic indices were recorded, showing toxicity at higher concentrations. Positive controls were functional. AKD-2023 treatment in the absence and presence of S9 Mix did not increase the incidence of chromosomal aberrations under study conditions. **No adverse effect**. **ACCEPTABLE**. (Kishiyama and Gee, 8/1/02).

DNA DAMAGE

** 031 184425 Proudlock, R. "AKD-2023 Technical Mouse Micronucleus Test." (Huntingdon Research Centre Ltd., Laboratory Project ID: AGK 15/930500, April 14, 1993.) AKD-2023 Technical (96.5%) was tested at 0 (0.5% CMC), 1250, 2500 or 5000 mg/kg, administered once via gavage at 20 ml/kg to 15 CD-1 mice/sex/group with 5/sex/sacrifice time (24, 48 and 72 hours post-dosing). AKD-2023 treatment did not induce a statistically significant or dose related increase in the number of micronucleated polychromatic erythrocytes. An equivocal cytotoxic effect was noted at 72 hours post-dosing with AKD-2023 treatment in terms of a decrease in the ratio of polychromatic erythrocytes/normochromatic erythrocytes. Clinical signs of piloerection and hunched position were seen at all doses in the first 24 hours with the severity slightly increased at 5000 mg/kg. Although dosing solutions were not analyzed, the study was evaluated as **ACCEPTABLE** based on clinical signs. **No adverse effect** under study conditions. (Kishiyama and Gee, 8/2/02)

METABOLISM

As noted below, the following two studies address the metabolism data requirements.

52893-032 184426 Mayo, B. C., "^{14}C-AKD 2023: metabolism in the rat," Huntingdon Life Sciences Ltd., Cambridgeshire, England, 7/7/97. Lab Project ID # AGK 22/952284. Small groups of CD rats were dosed orally with acequinocyl, labeled with ^{14}C in the phenyl or dodecyl

moieties, using 1% methyl cellulose as vehicle. Both label placements were involved in single low dose studies (10 mg/kg). High dose (500 mg/kg) and 14-day repeat-dose studies (10 mg/kg/day) were performed with phenyl-labeled acequinocyl only. Initial studies using CO_2 traps found less than 0.2% of dose in exhaled air. Absorption was about 25-42%, based on bile duct cannulation studies, which found 20-33% of administered dose in bile, plus 5-9% in urine plus cage wash. Intact rats excreted most of the dose in feces, compared with less than 15% of label being found in urine. Clearance from the body was rapid, with reductions in most tissue levels at least 10-fold between 24 hr and 72 hr post-dosing. Parent compound was not detectable in urine, and was only a minor component (typically 1-2%) of fecal residues. Cleavage of the acetyl group of acequinocyl yielded 2-hydroxy-3-dodecyl-1,4-naphthalenedione (designated "R1") as a major fecal metabolite (12-36% of dose). Subsequent oxidation of the dodecyl chain yielded butanoic (AKM 14) and hexanoic (AKM 15) acids, the only measurable identified urinary metabolites. An apparent final product of epoxidation of R1 was AKM 18, [2-(1,2-dioxotetradecyl)-benzoic acid], which comprised 19-40% of label in feces. All four identified metabolites were found in bile and plasma in similar proportions. There were no remarkable differences in metabolite disposition due to gender, and no effect of pre-dosing for 2 weeks. The large dose slowed transit time and appreciably reduced absorption. This study is the primary record on pharmacokinetics and tissue distribution, while another record (Record No. 184427 in Document No. 52893-033) specifically serves metabolite identification. Aldous, 8/15/02.

52893-033 184427 Maki, S. and Y. Kato, "AKD-2023: identification and quantification of metabolites in rat urine and bile," Institute of Environmental Toxicology, Tokyo, 7/28/97. Lab Project ID #: IET 96-8010. This study utilized 5 male CD rats, 3 of which were cannulated to obtain bile samples. Bile samples were evaluated for metabolite composition from 12 rats used in Record No. 184426, including 3 rats/sex/treatment at low and high dose levels (10 and 500 mg/kg single dose treatments, respectively). The present study confirmed that the only two major urinary metabolites were AKM 14 and hexanoic AKM 15, consistent with results from Record No. 184426. The present study identified the major biliary metabolite as conjugate of AKM-05 [the same molecule designated "R1" in Record No. 184426, i.e. de-acetylated parent compound]. The conjugation moiety is presumed to be glucuronic acid, based on the MW of the conjugated product. Together, Record Nos. 184426 and 184427 provide the essential information needed to fill the metabolism data requirements for acequinocyl. Aldous, 8/16/02.

52893-033 1844278 Kemp, L., "^{14}C-AKD 2023: Percutaneous absorption in the rat (*in vivo*) method," 7/20/2000. This study summary indicates that acequinocyl is only sparingly absorbed through intact skin. This study type is evaluated by Worker Health and Safety Branch.

SUBCHRONIC STUDIES

(Oral)

023; 184413; "AKD-2023 Technical: Subchronic Feeding Study in Rats" (Inoue, H., Biosafety Research Center, Foods, Drugs and Pesticides (An-Pyo Center), Shizuoka, Japan, Laboratory Project ID: Experiment No. 2183 (154-004) Report No. 2742, 2/9/98). 870.31. AKD-2023 Technical (Lot No. AK23921T, purity = 96.5%) was admixed to the feed and fed to 10 F344/DuCrj (Fischer) rats per sex per dose at dose levels of 0 (basal diet only), 100, 400, 1600, or 3200 ppm (0, 7.57, 30.4, 120, 253 mg/kg/day, respectively for males and 0, 8.27, 32.2, 129, 286 mg/kg/day, respectively for females) for 13 consecutive weeks. All animals at 3200 ppm died during the first 3 weeks of the study; no other mortalities occurred. Treatment-related reddish urine was observed in both sexes at 1600 ppm from Week 2 through Week 13. Hematology revealed a treatment-related increase in mean white blood cells and platelets in males at 1600 ppm with a treatment-related increase in mean prothrombin time and activated

partial thromboplastin time in both males and females at 1600 ppm. Urinalysis revealed treatment-related red-brown urine in all animals of both sexes at 1600 which is consistent with the increased number of animals with bilirubin in their urine. Treatment-related yellow-brown urine in all animals of both sexes at 400 ppm suggesting the presence of the metabolite of the test material. Macroscopic examination on the surviving animals revealed an enlarged eye in 2 females at 1600 ppm. Microscopic examination on the surviving animals revealed no treatment-related abnormalities. Macroscopic and microscopic examinations of the mortalities (including animals in moribund condition) revealed hemorrhaging of muscle and other organs and wasting of the whole body at 1600 and 3200 ppm dose levels. **Possible adverse effect:** inhibition of the coagulation system. NOEL (M)= 7.57 mg/kg/day (100 ppm) and NOEL (F) = 8.27 mg/kg/day (100 ppm) based on the presence of yellow-brown colored urine. **Acceptable**. (Corlett, 6/28/02)

036; 187914; "AKD-2023 Technical: Toxicity Study in Mice by Dietary Administration for 13 Weeks" (Waterson, L.A., Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England, Laboratory Project ID: AGK 19/931803, 1/16/95). 870.3100. AKD-2023 Technical (Lot No. AK 23921T, purity = 96.5%) was admixed to the diet and fed to 20 Crl: CD-1(ICR)BR mice per sex per dose at dose levels of 0 (untreated diet), 100, 500, 1000, or 1500 ppm (0, 16, 81, 151, 295 mg/kg/day, respectively for males and 0, 21, 100, 231, 342 mg/kg/day, respectively for females) for 13 weeks. All animals at 1500 ppm either died or were sacrificed due to poor condition by Day 4 of Week 2. Also, 8 females and 1 male at 1000 ppm were either found dead or sacrificed due to poor condition during the first 3 weeks of the study and 1 male at 500 ppm was found dead during Week 11. Dose-related pallor/pale extremities, orange stained urine, sunken eyes, piloerection, and labored/irregular breathing were observed at 1000 and 1500 ppm in both sexes. In animals that survived to terminal sacrifice, a treatment-related increase in mean relative liver weight in both sexes at 500 and 1000 ppm was observed together with treatment-related histopathological findings that included centrilobular hepatocyte vacuolation (in both sexes at 500 and 1000 ppm), generalized hepatocyte vacuolation (in males at 1000 ppm and in females at 500 and 1000 ppm), and centrilobular hepatocyte enlargement (in females at 500 and 1000 ppm). **No adverse effects** (except for mortality at high dose level (males) and at medium-high and high dose levels (females)). NOEL (M) = 16 mg/kg/day (100 ppm), NOEL (F) = 21 mg/kg/day (100 ppm) based on increased mean relative liver weight and liver histopathology. **Acceptable**. (Corlett, 8/26/02)

024; 184414; "AKD-2023 Technical: Toxicity to Dogs by Repeated Oral Administration for 13 Weeks" (Waterson, L.A., Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England, Laboratory Project ID: AGK 18/932543, 1/16/95). 870.3150. AKD-2023 Technical (Lot No. AK 23921T, purity = 97.1%) was administered via gelatin capsules to 4 pure-bred beagle dogs per sex per dose at dose levels of 0 (empty capsules), 40, 160, 640, or 1000 mg/kg/day once a day 7 days per week for 13 weeks. All animals at 1000 mg/kg/day (one male during Week 2 and the others on Day 3 of Week 3) and 2 females at 640 mg/kg/day (one during Week 3 and one during Week 4) were sacrificed due to inappetence and body weight loss. No other deaths occurred during the study. Dose-related colored feces (red, red/orange, or red/brown) were observed at 40, 160, and 640 mg/kg/day in both sexes. A treatment-related decrease in body weight gain in males at 160 and 640 mg/kg/day was observed. Hematology revealed a treatment-related increase in mean platelet count in females at 160 and 640 mg/kg/day. Macroscopic and microscopic examinations on the surviving animals revealed no treatment-related abnormalities. Microscopic examinations of the animals sacrificed intercurrently revealed reduced cellularity and congestion of the bone marrow in the spleen. NOEL (M/F) = 40 mg/kg/day based on decreased mean body weight gain. **Acceptable**. (Corlett, 7/10/02)

(Dermal)

025; 184415; “AKD-2023: 28 Day Dermal Administration Toxicity Study in the Rat with a 14 Day Treatment-free Period” (Chowdhury, P., Covance Laboratories, Harrogate, North Yorkshire, England, Laboratory Project ID: CLE Study Number 619/48, 7/30/99). 870.3200. AKD-2023 Technical (Branch Number AK23931T, purity = 97.1%) was made into a paste with 1% methylcellulose (w/v) and applied to the clipped dorsal skin of 10 Crl:(IGS)CD BR rats per sex per dose at dose levels of 0 (vehicle only), 40, 200, or 1000 mg/kg/day (plus an additional 10 animals per sex per dose at 0 and 1000 mg/kg/day to assess recovery for 2 weeks) for 6 hours per day 7 days a week for at least 28 days using a semi-occlusive dressing. No treatment-related mortalities occurred. Red staining on the back of all animals at 40, 200, and 1000 mg/kg/day was observed in the morning only, from Day 2 through Day 13; the number of animals exhibiting this sign decreased in a dose-related manner after Day 13. The biological significance of this sign is unknown at this time. Hematological investigations revealed treatment-related increases in mean prothrombin time (in males only), activated partial thromboplastin time (in males and females), and fibrinogen (in males only) levels at 1000 mg/kg/day. Macroscopic and microscopic examinations revealed no treatment-related abnormalities. **No adverse effects.** NOEL (M/F, systemic) = 200 mg/kg/day based on blood clotting parameters. NOEL (M/F, skin) = 1000 mg/kg/day based on no treatment-related effects at the highest dose tested. **Acceptable**. (Corlett, 7/16/02)